PB86-205598

Systems and Results of Tests for Chemical Induction of Mitotic Malsegregation and Aneuploidy in 'Aspergillus nidulans'

Oak Ridge National Lab., TN

Prepared for

Environmental Protection Agency, Washington, DC

1986

U.S. Department of Commerce
National Technical Information Service
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TECHNICAL REPORT DATA
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1. REPORT NO. EPA/600/J-86/075	2. PB86-205598
4. TITLE AND SUBTITLE Systems and Results of Tests for Chemical Induction of Mitotic Malsegregation and Aneuploidy in Aspergillus nidulans (Journal Version)	5. REPORT DATE 1986 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Etta Käfer, McGill University; Barry R. Scott, Phoenix Lone Oak Lab.; Andreas Kappas, Nuclear Rsch. Ctr, Athens	8. PERFORMING ORGANIZATION REPORT NO. -
9. PERFORMING ORGANIZATION NAME AND ADDRESS Oak Ridge National Laboratory Env. Mutagen, Carcinogen & Teratogen Info. Program Oak Ridge, TN 37830	10. PROGRAM ELEMENT NO. 11. CONTRACT/GRANT NO. IAG#DW899-30922 Contract#68-02-3839
12. SPONSORING AGENCY NAME AND ADDRESS Office of Health & Environmental Assessment (RD-689) Reproductive Effects Assessment Group U.S. Environmental Protection Agency Washington, D.C. 20460	13. TYPE OF REPORT AND PERIOD COVERED Final 14. SPONSORING AGENCY CODE EPA/600/21

15. SUPPLEMENTARY NOTES
One of 12 articles prepared by a committee and including an introductory and summary paper. These reports were published together in Mutation Research, 167 (1986).
Mutation Res., 167:9-34, January 1986

16. ABSTRACT

A committee of scientists was established by the U.S. Environmental Protection Agency to appraise the current state of aneuploidy test methodology, to compile and analyze published data on the chemical induction of aneuploidy, and to provide guidance for additional test development and validation. The committee was organized into 9 working groups, representing different eukaryotic organisms ranging from fungi to whole mammals. The Environmental Mutagen, Carcinogen, and Teratogen Information Program at Oak Ridge National Laboratory identified and provided literature to the working groups. The 9 reports were prepared by the individual working groups. The reports include: (1) descriptions of the available testing approaches and general principles to be followed for protocol development, (2) criteria for evaluating data, and (3) recommendations for future test development and basic research. The evaluated results are presented in tables. For the positive and negative calls, the lowest effective dose tested or the highest ineffective dose tested, respectively, and the range of concentrations tested are provided. An overall summary report was prepared that summarizes the conclusions and recommendations of the 9 working groups and the chemical data base. It is hoped that these reports will provide a framework for further test development and validation of aneuploidy assays.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT Release to the public	19. SECURITY CLASS *(This Report)* Unclassified	21. NO. OF PAGES 28
	20. SECURITY CLASS *(This page)* Unclassified	22. PRICE

EPA Form 2220-1 (9-73)

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EPA/600/J-86/075
JOURNAL ARTICLE

Mutation Research, 167 (1986) 9–34
Elsevier

MTR 05003

Systems and results of tests for chemical induction of mitotic malsegregation and aneuploidy in *Aspergillus nidulans* *

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(Received 14 June 1985)
(Accepted 27 June 1985)

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* Report of the Aneuploidy Data Review Committee (Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC).

The review described in this article has been funded by the U.S. Environmental Protection Agency through Contract No. 68-02-3839 and through an interagency agreement (No. DW899-30922) to the Oak Ridge National Laboratory. It has not been subjected to Agency review and therefore does not necessarily reflect the views of the Agency and no official endorsement should be inferred.

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Abbreviations: CIPC, chlorpropham (isopropyl-3-chorophenyl-carbamate); CTT, 4-chloro-trifluorotoluene; DMSO, dimethyl sulfoxide; DNCTT, 4-chloro-3,5-dinitrotrifluorotoluene; DTFB, 4,5-dichloro-trifluoromethylbenzimidazole; FUDR, 5-fluorodeoxyuridine; MBC, methyl-2-benzimidazole carbamate; MMC, mitomycin C; MMS, methyl methanesulfonate; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; NCTT, 4-chloro-3-nitrotrifluorotoluene; 4NQO, 4-nitroquinoline-*N*-oxide; PFPA, *p*-fluorophenylalanine; SD, sodium deoxycholate; TTFB, 4,5,6-trichloro-2-trifluoromethylbenzimidazole; 2*n*-N and 2*n*-CO, diploid segregants which show homozygosis for genetic markers as produced by nondisjunction (N) or crossing-over (CO).

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Summary

In Aspergillus several types of test systems have been developed for detection of chemicals which induce aneuploidy and/or malsegregation of chromosomes. Results from 23 papers were reviewed in which numerical data for 42 chemicals had been reported. The test systems fall into two groups. One group includes all purely genetic tests that detect euploid mitotic segregants from heterozygous diploids and identify these either as products of malsegregation of chromosomes or as products of crossing-over (13 papers, several reviewed in detail previously; Käfer et al. (1982) and Scott et al. (1982)). The other group includes tests that treat haploid or diploid strains and detect aneuploids as unstable abnormally growing segregants which can be identified as specific disomics or trisomics by their characteristic phenotypes. In addition, such tests characterize abnormal segregants from heterozygous diploids by correlating phenotypes with patterns of genetic segregation in spontaneous euploid sectors. This analysis makes it possible to distinguish between induced primary aneuploidy of whole chromosomes and partial tri- or monosomy resulting from chromosome breakage and secondary spontaneous malsegregation (10 papers).

Based on results of both types of tests, it is postulated that chemicals which cause increases of euploid malsegregants, but not of crossovers, normally induce aneuploids as primary products (as shown for 7 of the 14 cases). These include compounds which damage spindles or membranes (especially the well-known haploidizing agents) and generally are effective only when growing cells are exposed. (8 chemicals that may belong in this category could not be classified for certain, because information was insufficient.) On the other hand, chemicals which cause increases of all types of euploid segregants (11 cases), mostly induce drastic mutations and aberrations as primary effects and cause spontaneous malsegregation or crossing-over only as secondary events (as demonstrated for radiation-induced abnormals). In addition, a few chemicals were negative, because they increased only crossing-over or showed no increased segregation at all at concentrations which reduced survival or growth rate (9 cases). Recommendations are made for standardization of methods and protocols. New tester strains and specific procedures are outlined which should be useful for conclusive tests of chemicals that may induce aneuploidy.

(1) Introduction

(1.1) Relationship of current and previous reviews

The fungus *Aspergillus nidulans* has been used for many years to test potential environmental mutagens for a variety of genotoxic effects. Using suitably marked heterozygous diploids, induced mitotic crossing-over and chromosome malsegregation have been identified after treatment with radiation or various chemicals. The latter process often is assumed to be due to nondisjuction, and aneuploid intermediates are postulated to have been induced.

Investigations of this type that were published before 1980 have been reviewed as part of the U.S. Environmental Protection Agency Gene-Tox Program (Käfer et al., 1982). A summary of all tested chemicals and obtained results is included in the review of tests for effects on mutagenicity (Scott et al., 1982). Thirteen of the publications included in these reviews provided data relevant for the question of chemically induced aneuploidy. These data will be discussed but not presented in the current review which covers mainly papers published 1979–1984 (a few of the most recent ones were not available in time and are only mentioned briefly at the end). Since the basic premises, genetic basis, and methods of these early tests have been characterized in detail in the 1982 reviews, any additional tests which use the same methods (2 papers) will be summarized briefly, while modifications of the described test systems (3 papers), or new approaches which attempt to give more conclusive evidence for induced aneuploidy (5 papers), will be characterized in detail. Only chemicals for which numerical data are available have been included. This unfortunately eliminates most of the negative cases.

(1.2) Basic characteristics of mitotic segregation and aneuploids in Aspergillus

A. nidulans, a haploid homothallic ascomycete, occasionally shows nuclear fusion in vegetative cells. The resulting diploids are fairly stable, but meiotically sterile. If marked haploid strains are combined into forced heterokaryons, diploids of any desired constitution can be obtained (Roper, 1952). Such diploids show rare events of spontaneous mitotic recombination which produces 3 types of euploid segregants. The first of these are diploid recombinants which result from mitotic crossing-over (Pontecorvo et al., 1953). Reciprocal products of single events can be recovered from suitably marked strains as 'twin spots' (i.e., pairs of complementary types, Wood and Käfer, 1968). In addition, more rarely, Aspergillus diploids produce haploid segregants, thus completing the parasexual cycle (Pontecorvo, 1956; Roper, 1971). Haploids are the results of malsegregation of whole chromosomes. This process also produces the third type of euploid segregants, namely, diploids homozygous for all markers of one or more chromosomes (Pontecorvo and Käfer, 1958).

The spontaneous events of chromosome malsegregation can be analyzed in diploids which carry genetic markers on all homologues (8 pairs; Käfer, 1958, 1961). Haploids probably originate from aneuploids, since hyperhaploids have been found more frequently than true haploids among conidia of heterozygous diploids. Both types are relatively rare (haploids $< 0.02\%$). Aneuploids of any type are regularly found (usually 1–2%). However, most of these are hyperdiploids, trisomic for few chromosomes, usually one or sometimes 2 or 3 ($2n + 1$ or 2 or 3). In addition, in a small fraction of cases (10^{-4}), spontaneous haploids seem to arise as the product of extensive simultaneous malsegregation (i.e., breakdown of mitosis). In contrast, such general maldistribution of chromosomes is always found when segregants are induced (e.g., by 'haploidizing' agents which interfere with the function of the spindle).

All aneuploids grow and conidiate less well than normal euploid strains, but only types with one extra chromosome have been recovered regularly as separate abnormal colonies. These show various phenotypes, but all are unstable, and they are easily outgrown by euploid segregants which are produced by secondary nondisjuction or chromosome loss. The resulting characteristic and recognizable aneuploid phenotypes are specific for each of the 8 disomic ($n + 1$) and trisomic ($2n + 1$) types (Käfer and Upshall, 1973).

All evidence available indicates, that *spontaneous* aneuploids arise from mitotic nondisjunction of single chromosomes. In the first instance,

this produces $2n + 1$ and $2n - 1$ nuclei from diploid ones. The latter apparently are extremely inviable but presumably give rise to the much rarer hyperhaploid ($n + 1$ to 3) and haploid types. Any $n + 2$ or 3 aneuploids, as well as rare $2n + 2$ or 3 types, produce haploid or diploid secondary segregants only in stepwise fashion as the final product of increasingly prominent and faster-growing sectors (Käfer, 1961). Each step is correlated with segregation of heterozygous markers of a single chromosome. Several $n + 1$ sectors can be recognized, permitting visual classification of most hyperhaploid types (Käfer, 1985). While some of the $2n + 2$ types have typical phenotypes, most are not easy to distinguish. Triploid strains produce a wealth of subtly varying hyperdiploid types which defy visual identification, except when typical $2n + 1$ segregants are obtained in replatings (Käfer, 1977).

Spontaneous mitotic segregants in Aspergillus diploids originate, therefore, from two basic types of genetic recombination: (1) Diploid recombinants resulting from crossing-over which are homozygous for all or some of the markers of one chromosome arm and show normal viability similar to the parental diploid type (i.e. the colonies remain normal-looking). (2) Products of chromosome malsegregation which, in the first instance, are aneuploid nuclei that persist in heterokaryotic condition in the mycelium; these occasionally show secondary nondisjuction or chromosome loss and the most viable types form conidia. However, only their euploid end-products produce patches or sectors large enough to be detectable without a microscope. The latter are either haploids, or so-called nondisjunctional diploids which are homozygous for all markers of any segregating chromosome. Therefore, while pure aneuploids always look abnormal, their secondary euploid segregants actually may be found as small segments in 'normal' colonies.

These general rules do not apply when chromosomal aberrations are present. For example, in diploids heterozygous for reciprocal translocations, even mitotic crossing-over can produce unstable segregants which are partially tri- and monosomic, unbalanced, types. These resemble aneuploids, grow poorly, and produce euploid better-growing sectors by further segregation. Mitotic crossing-over is considerably more frequent than nondisjuction in untreated diploids. It follows that in translocation heterozygotes most abnormals produce normal sectors by spontaneous crossing-over rather than by chromosome malsegregation (Käfer, 1976). Furthermore, diploids heterozygous for deletions, or haploids with duplications, also look abnormal and occasionally produce euploid sectors by various mechanisms (Käfer, 1963; Roper, 1971). Not surprisingly, large duplications may produce phenotypes that are very similar to those of aneuploids disomic for the chromosome of the duplication.

These findings make it obvious that abnormal phenotypes (which are always environmentally modulated) can reflect several types of genetic abnormalities. Stable abnormal types, which are usually very rare in controls, are due to mutation. Unstable sectoring abnormals in untreated standard diploids are practically always aneuploids, but treated cultures produce two types of unstable abnormals: (1) whole-chromosome aneuploids showing the expected segregation patterns, or (2) partial aneuploids producing euploid sectors by crossing-over or by malsegregation of whole chromosomes.

In partial aneuploids, the subsequent segregation is a spontaneous secondary event. It represents the response of the vegetative nuclei to the induced damage and the production of visible sectors reflects the relative selective advantage of euploid types. Such competitive relationships between aneuploid and euploid types seem to be highly species specific. For example, the tolerance for aneuploidy in yeast is much higher than in Aspergillus. Therefore, while the primary effects may occur with similar frequencies in all species, the secondary response is expected to be quite different in different test organisms. The specific features of Aspergillus (e.g., sectoring aneuploids which are not found in Neurospora or yeast) not only depend on the relative viabilities of different types, but also on cellular characteristics which affect selective multiplication (e.g., the occurrence and relative instability of heterokaryons). Furthermore, the high frequency of secondary nondisjunction in nuclei which are heterozygous for aberrations may be unique to species which show chromosome pairing and crossing-over in vegetative

cells (ascomycetes and diptera, e.g., Drosophila). The question arises, whether the unusual response of such somatic cells may be usable as a model for the meiotic situation.

(2) Test description

Three basically different types of test systems have been developed in *A. nidulans* to assess effects on chromosomal segregation or on induction of aneuploids in vegetative nuclei. (1) Systems which analyze euploid segregants from treated heterozygous diploids to identify those types which are homo- or hemizygous for all markers on both arms of a well-marked chromosome. Many different diploids, different methods of treatment, and different procedures for isolation or selection of segregants have been used for such tests. (2) Systems which analyze samples of unstable abnormal segregants from well-marked heterozygous diploids to identify aneuploid products by their patterns of genetic segregation in euploid sectors. (3) Tests which identify aneuploids among abnormal colonies from treated haploid strains by their characteristic phenotypes.

(2.1) Genetic basis of effect detected

All 3 types of tests are based on mitotic recombination and segregation in vegetative cells of Aspergillus (Käfer et al., 1982). However, their aims are not identical. Methods of the first type are designed to detect, in relatively simple tests, any type of genotoxic effect that increases mitotic segregants. Methods of the other two types aim at providing evidence about primary effects of chemicals, by discriminating between cases in which aneuploids have been induced and cases in which breakage or mutation leads to secondary malsegregation.

The stated aim of the first type of investigation often is to identify whether treatment increases the occurrence of one of the two spontaneous processes, either mitotic crossing-over or nondisjuction. However, it has become clear that the induced effects often are qualitatively different and do not represent simple increases in spontaneous segregation. This may not generally be obvious because the final euploid segregants are always of the same three types as found spontaneously (namely diploid and haploid products of malsegregation ($2n$-N and n types) and crossover diploids ($2n$-CO)). However, the *primary* induced effects are of at least 4 basic types. Two are similar to the two spontaneous events, namely (i) mitotic crossing-over or (ii) very rarely, nondisjuction of single chromosomes; and two are of clearly different types which spontaneously occur only at extremely low frequencies, namely (iii) gross malfunction of the division process which results in extensive chromosome malsegregation, or (iv) drastic mutations, which have semidominant effects and cause reduced growth rate but can be eliminated by spontaneous mitotic recombination or segregation.

These consideratons make it evident that test systems of the first type which measure overall frequencies of euploid segregants are only able to demonstrate increases of certain segregants caused by a specific chemical, but cannot prove that a particular type of segregation was induced. The obtained segregants might well be the result of spontaneous secondary responses of the damaged nuclei.

The second and third methods try to obtain evidence that aneuploids are actually induced. They attempt to answer the question whether whole-chromosome malsegregation is a primary induced effect of the agent in question, or whether some other type of damage to genomes secondarily leads to selection of spontaneous chromosomal segregants. The second method simultaneously identifies induced crossing-over, distinct from induced chromosome damage followed by selection of spontaneous crossovers.

(2.1.1) Tests which analyze euploid segregants from heterozygous diploids

In this type of test, suitably marked diploids (Section 2.2) are treated, and segregants are identified after growth on agar media. Two main techniques have been used that differ in the method for isolation of euploid segregants. One type is selective and uses special media and recessive resistance markers to isolate solely the segregants of interest. The other type identifies color segregants in all surviving colonies on nonselective media.

The selective methods have the advantage that relatively few colonies have to be obtained and

that among these, two (or all three) of the euploid segregant types can be identified visually by color. However, the stringent selective conditions affect recovery and modify the relative frequencies of different types. Generally, only the most viable types can compete and, among these, diploid crossovers are especially favored. In contrast, products of chromosome malsegregation are relatively rare and aneuploids are hardly ever recovered, but haploids compete relatively well and may represent an unusually high fraction (de Bertoldi et al., 1980). A further disadvantage of these systems is the finding that the selective agents used are potent inducers of aneuploidy themselves. More recently they have, therefore, been used mainly to detect general genotoxic effects and especially to measure levels of induced mitotic crossing-over (e.g., Gualandi et al., 1979).

Analysis by nonselective methods involves more steps and larger samples. Diploids heterozygous for suitable color mutants are grown on media which support growth of all possible types (usually complete medium). In all cases color segregants will need to be tested for additional biochemical markers to distinguish between the 3 possible euploid types. If only minor effects are observed, a very large number of plates and colonies may be needed to demonstrate or rule out any induced effects. Some investigators have, therefore, resorted to media containing deoxycholate, which reduces colony size (Mackintosh and Pritchard, 1963). However, this agent has recently been shown to induce aneuploidy, especially at lower temperatures (25–30°C; Assinder and Upshall, 1982; Table 2).

More conclusive results are obtained when conidia are plated on standard media on which it is possible to distinguish abnormal, potentially aneuploid, colonies from normal euploid ones. Color segregants may be obtained from both, but induced crossing-over results in normal colonies with large color segments, including twin sectors, while induced aneuploidy produces abnormal colonies with color sectors that show whole-chromosome segregation. In these cases information about the relative growth rates of primary green colonies and their color segregants gives additional weight to the clear-cut but indirect genetic evidence. In contrast, if all 3 types of euploid segregants are increased, the genetic evidence is ambiguous. In this case, the details on relative rates of growth will be not only helpful, but crucial, for deciding between two possible explanations: (i) induction of both types of segregation, when color sectors in normal colonies are mainly the result of crossing-over and sectors of abnormal colonies are mainly products of malsegregation; or (ii) induced aberrations when euploid color segregants of normal as well as abnormal colonies are of all 3 possible types.

In either case, more conclusive evidence will be needed and samples of treated survivors will have to be analyzed in detail. In cases of apparent crossover types analysis by haploidization has shown that even these can be deceiving and may represent small terminal deletions (Kappas, 1978). Detailed analysis of apparent aneuploids is crucial to distinguish agents which induce aneuploids as primary effects from those that cause defects leading to secondary malsegregation. In Aspergillus, the following methods are used to make such distinctions.

(2.1.2) Tests which identify aneuploids among abnormals from treated diploids

Many agents cause production of abnormal colonies. Aneuploid abnormals are very unstable and produce diploid and/or haploid sectors by chromosome loss or malsegregation. A slight increase of crossing-over, not correlated with production of sectors, may be observed, because aneuploids seem to show higher spontaneous crossing-over than diploids (Käfer, 1977; Upshall et al., 1979). Confirmation of aneuploidy can be obtained if a sample of cases is analyzed in detail. Abnormal centers are replated (rather than transferred), the resulting secondary types inspected, and some of their sectors tested. In all cases, some $2n + 1$ and/or $n + 1$ types with specific phenotypes that correspond to the genetic segregation should be recognizable (Käfer and Upshall, 1973). If marker segregation confirms loss of whole chromosomes, and especially if low levels of treatment produced recognizable trisomics in the original platings, induced aneuploidy can safely be postulated.

Frequently, however, induced malsegregation is extensive and complex hyperdiploid types may defy visual classification, especially if chemicals

are present in agar media and act continuously during growth. In such cases, simple $2n + 1$ may be rare even in replatings of abnormals and may only be found if two or more steps of replating are used, as demonstrated for some complex types induced by ethanol (Käfer, 1984). In such cases, loss of a chromosome sometimes may not correlate with segregation of heterozygous markers, presumably because all homologues are identical.

In such complex situations, the most economical method is to concentrate on analysis of abnormal types which segregate for color markers on well-marked chromosomes. The resulting genetic information will be especially useful for identification of complicated abnormals which are not aneuploids. A first indication that they might contain semidominant lethals usually is that better-growing sectors are produced by crossing-over (i.e., segregation of markers on only one chromosome arm). While in normal diploids crossing-over has no effect on viability or growth rate, in diploids heterozygous for semidominant lethals (e.g., terminal deletions) crossing-over can eliminate such mutations and produce better-growing segregants homozygous for the chromosome arm of the unaffected opposite homologue. A second criterion is the finding that every abnormal survivor is different from all others, each being the result of a different induced mutation. Detailed analysis of a fairly small sample of cases, which segregate for a color mutant on a well-marked chromosome, will demonstrate a variety of patterns of segregation that clearly differ from each other and from those of standard trisomics. For example, some of the aberration types with yellow sectors may produce homozygous *yA* segregants by crossing-over as well as by malsegregation (presumably if mutation or breakage occurred on the yA^+ chromosome arm). Others may produce yellow sectors exclusively by nondisjunction, but not show the 2:1 ratio for parental green ($yA/+$) to yellow (yA/yA) diploid types typical for trisomics. In fact, frequently no normal green sectors are found, or only rare cases resulting from two steps of segregation (as expected when the yA^+ arm carries a distal, semidominant, lethal).

(2.1.3) Tests which visually identify aneuploids from treated haploids

The preceding tests with diploids can give evidence for induced aberrations but cannot rule out low levels of induced aneuploidy. A few abnormals usually are found which are indistinguishable from trisomics. As a more conclusive test, it seems worthwhile to treat haploid conidia and to identify frequencies of hyperhaploids. Chromosome breakage, which leads to deletions and other unbalanced aberrations, is likely to be lethal for haploid conidia and abnormal sectoring types are unlikely to be cases of secondary chromosome malsegregation. Induced aneuploidy in haploids is relatively conclusive evidence of a primary event. A disadvantage is that such aneuploids do not segregate for markers and can be recognized only visually. On replating, however, any complex hyperhaploids will produce some $n + 1$ disomics, which are relatively easy to recognize (Käfer, 1961). Because of phenotypic variation caused by variable crowding or different depth of agar media, all 8 types may not be identifiable individually. However, some have very unique appearances and are usually recognizable (especially n + VI and n + VII types, i.e., $n + 1$, disomic for chromosomes VI or VII). Other pairs may not be distinguishable and may need to be recorded together (n + I or n + III, and n + IV or V, and n + II or VIII).

More complex types will be encountered if agents induce polyploidy. In such cases, hyperdiploids are obtained which have less distinct phenotypes. With experience, visual identification of their $2n$ sectors, and of induced $2n$ colonies, is quite feasible (Käfer, 1977). These may be confirmed by haploidization on benlate media (Upshall et al., 1977) or by measurements of conidial diameters (Pontecorvo et al., 1953). Detection of induced polyploidy is more difficult with diploids, because $3n$ and $4n$ types cannot be identified visually, nor conclusively be demonstrated by genetic analysis.

Such tests with haploid strains are obviously complementary to the genetic analysis of euploid sectors from treated diploids. They depend a great deal on experimental details which influence phenotypes and on a certain amount of experience with simple disomics and their relative frequencies of sectoring (Käfer and Upshall, 1973). They are especially meaningful when results are positive

Fig. 1. Genotypes of recently constructed test diploids with markers in linkage groups I–VIII (for symbols and map distances see Clutterbuck, 1982, with two exceptions: *pimB* = recessive pimaricin resistance in diploid **23**, de Bertoldi et al., 1980; *ssbA* = suppressor of *sbA* in diploid **26**, Section 2.2.1). Component strains for diploid **22** (used by Upshall and coworkers, Table 2) and for **29** and **30** (Käfer, 1985) are available from FGSC (Fungal Genetics Stock Center No. as indicated).

(i.e., when unstable, sectoring abnormals with expected aneuploid phenotypes are definitely obtained). In such cases, they are valuable for quick and conclusive identification of primary aneuploidy-inducing agents. Negative results are less useful, but help to indicate that mutation rather than aneuploidy was induced if stable abnormal types (i.e., morphological mutants) are encountered. Such types can be tested by mass-transfers to document that each has a unique phenotype and does not produce normal sectors (Käfer, 1985).

(2.1.4) Summary of tests, genetic basis and categories of assay codes

When one considers the type of information obtained by the various tests, the test systems fall into two groups. This leads to the two following basic assay codes (as shown in Table 6):

AN1: Tests which identify euploid mitotic segregants of various types.

AN2: Tests which identify aneuploids.

As described above, different methods have been developed for each of these, some of them suited to special situations. In addition, various tests have produced incomplete information, either because they used strains or procedures that cannot identify all types of segregants, or because they used short-cuts in recording or reporting of results. Such modifications of the test systems can be usefully expressed as subcategories of the assay code in the following way.

AN 1: Tests of euploid mitotic segregants:
- 1a: Non-selective; all 3 types identified (i.e., n, $2n$-N and $2n$-CO).
- 1b: Non-selective; only 1 or 2 types distinguished (or reported).
- 1c: Selective; modified ratios or number of types obtained.

AN 2: Tests for mitotic aneuploids:
- 2a: Abnormals replated; aneuploids identified by genetic segregation and/or phenotypic characteristics (the latter if haploids are treated).
- 2b: Aneuploids inferred from genetic segregation in euploid sectors of abnormal colonies.

(2.2) Description of strains and uses

(2.2.1) Genotypes and special features of test diploids

Most strains used and their specific features have previously been described in detail (Käfer et al., 1982). The few that have been constructed more recently are shown in Fig. 1 (diploids **22–30**).

Diploid **23** of de Bertoldi et al. (1980) is designed to select segregants homozygous for a marker on the left arm of chromosome I, namely *pimA*, a recessive mutant resistant to pimaricin (Section 2.1.1). Diploid **23** works on the same principle as the *fpaA*/+ diploids of Morpurgo and coworkers (Bignami et al., 1974, 1977). These are heterozygous for a recessive mutant conferring resistance to *p*-fluorophenylalanine (PFPA) (e.g., the frequently used diploid P1 which is shown as diploid **4** in Fig. 1 of Käfer et al., 1982). In both cases, the recessive resistance mutant is on the left arm of chromosome I and in coupling with *yA* (yellow conidia) on the right arm. Therefore, the selected resistant segregants are yellow if they result from chromosome malsegregation (either *yA* haploids or homozygous *yA*/*yA* diploids) and green (*yA*/+) if they are diploid mitotic crossovers. Unfortunately, the diploid strains used in these tests do not contain other color mutants, so that further analysis is necessary to distinguish between n and $2n$ types. Such tests are avoided in the *fpaA* system by selecting against haploid types, but this eliminates useful information (Table 1A and B). Therefore, various *fpaB*/+ diploids which can similarly be used in selective tests have been constructed (**19** or **20**, Käfer et al., 1982; and **29** or **30**, Fig. 1). These contain two further color mutants in repulsion on chromosome VIII (*chaA* and *fwA*), so that selected yellow segregants are always diploid while haploids are beige (either *yA;chaA* or *yA;fwA*).

In such selective tests, the concentration of the selective agent is very high and growth of any purely abnormal types, including aneuploids, apparently is inhibited. It seems likely that, at least in the *pimA* system, trisomics for chromosome I are not selected even when they contain 2 out of 3 resistant alleles, because these are recessive and trisomics relatively sensitive. Furthermore, monosomic $2n-1$ types, which are expected to be resistant half of the time, are known to be very

poorly viable in Aspergillus. However, the latter apparently are able to produce haploid nuclei fairly readily and haploids generally are found to predominate among chromosomal segregants, being 20 times more frequent than $2n$-N (de Bertoldi et al., 1980). Surprisingly, in spite of the known haploidizing effects of the selective agents in sensitive diploids when used at lower concentration, the ratios of crossovers to nondisjunctional resistant segregants are, in fact, in the normal range in the untreated samples (5 : 10 : 1). Presumably, fully resistant nuclei are impervious to the haploidizing effects of the selective agents, as shown for induction of abnormals by acriflavin in resistant *AcrA* strains (Ball and Roper, 1966). The main reservations concern the question of whether these agents might enhance or interfere with the effects of chemicals that are being tested. For this reason, results from selective tests are not included in several recent papers on induced nondisjuction (including the review of Morpurgo et al., 1979).

Other new diploids contain markers on all homologues, a feature lacking in the previously recommended diploids (**11**, and **19**; see feature 'e' in Figs. 1 and 2 of Käfer et al., 1982). Number **29** and **30** (Fig. 1) have been especially constructed for conclusive tests of aneuploidy (Käfer, 1985). They are similar to **26** (Käfer, 1984) with several improvements, including dominant resistance markers in coupling with recessives in linkage groups III and VII, and absence of *ssbA*, a suppressor of *sbA (ssbA* has also been found in FGSC strain No. 513, a component of diploid **20**; Käfer et al., 1982). Markers on all 16 chromosomes are essential when the aim is not only to show that abnormal colonies are aneuploid, but to rule out formation of better-growing sectors in abnormal colonies by mechanisms other than nondisjunction or chromosome loss (e.g., by crossing-over). Because the effort involved in testing for all markers is considerable, these diploids contain many optional markers which do not affect growth on minimal medium but can be tested if more information is desired (especially, markers that confer resistance or are unable to grow on unusual carbon sources).

The new diploids (**29** and **30**) also have the other desirable features listed in the text of Käfer et al. (1982):

(a) Markers to distinguish haploids from diploids (e.g., *suAadE, AcrA,* etc.).

(b) Markers to distinguish crossovers from chromosomal segregants for 1 or 2 chromosomes (namely, markers on the opposite arm from recessive color mutants, *yA* on chromosome I, and *wA* on II).

(c) Color markers in repulsion on the same chromosome arm which identify 'twin spots' as evidence for reciprocal crossing-over (*fwA* and *chaA* on VIII R).

(d) Many markers in both arms of one chromosome to follow segregation patterns after induction of aberrations (5–7 heterozygous in I in addition to *yA*).

(2.2.2) Construction of test diploids and procedures for verification of genotypes and for testing of segregants

In general, haploid strains are preserved as dry silica gel preparations which assures unchanged genotypes for many years (Scott and Käfer, 1982; Barratt et al., 1965, 1975). In addition, silica gels are conveniently prepared from the earliest pure culture of any synthesized diploid for which the genotype has been verified. When such samples are used their genotypes will automatically be rechecked, provided haploid segregants from untreated and positive controls are tested for segregation of markers.

For the original construction of diploids, haploid cultures are conveniently started on complete medium plates from a few grains of silica gel so that purity, phenotype, and growth rate can be checked. Using conidia from the appropriate pair of haploid strains, which carry different color and nutritional markers, forced heterokaryons are selected on minimal media using standard techniques (Pontecorvo et al., 1953; Clutterbuck, 1974; Scott and Käfer, 1982). From heterokaryons, stable green diploids are obtained, either as color sectors or as colonies from platings of conidia ($> 10^7$) in minimal medium.

To verify the diploid genotypes, samples are haploidized by transferring conidia to benlate media or benlate is used as a positive control (Hastie, 1970). The resulting haploids are tested for all markers at the same time as the parental haploid component strains and the isolated di-

ploid. The latter 3 strains are similarly used as controls on each 'master' plate, when segregants are replicated to test media. This facilitates classification, especially of the more variable resistance markers. When analysis is restricted to markers of chromosome I, these control strains are also added and supplements have to be included in test media for all nutritional mutants. Two sets of control media are conveniently used, one with all supplements, the other with only those of chromosome I that are being tested for. This procedure has the advantage that segregants homo- or hemizygous for nutritional markers of other linkage groups will be identified, and will not be excluded from test results because of the absence of growth factors.

(2.3) Suggested testing protocol

Details of the various useful tests and their problems have been discussed previously (Section IIC of Käfer et al., 1982). Some aspects, which also apply to mutagenicity assays, are described in the companion paper (Section IIC of Scott et al., 1982). Only a brief enumeration of the major steps is given here, except where recent experiences suggest modifications.

(2.3.1) Media

The media used for various tests are minor modifications of those devised by Pontecorvo et al. (1953). These, as well as most standard techniques, have been described in detail in Scott and Käfer (1982).

(2.3.2) Preparation of conidial suspensions

To obtain concentrated suspensions, strains are conveniently grown on solid media in flasks, and conidia collected by addition of liquid and gentle vortexing. The final suspension is prepared as described by Scott et al. (1982). The procedure includes removal of germination inhibitor by washing. However, experience shows that washing of conidia may not improve synchrony when pregerminated conidia are used for treatment, and washing at variable temperature may have the opposite effect (Käfer, 1984).

(2.3.3) Suggested exposure protocol

Details of the various exposure protocols have been outlined by Scott et al. (1982), where methods for metabolic activation in conjunction with exposure are also described (not discussed here).

(2.3.3.1) Exposure of mycelia during growth on media containing chemicals (= agar incorporation). This method is very useful for the detection of effects of stable chemicals on dividing nuclei. Transferring conidia to media with different concentrations permits easy identification of a suitable, slightly inhibitory, dose range. Recent tests showed that subsequent plating of conidia onto agar media which contain a range of the lower but inhibitory levels of chemicals can be a very appropriate method, especially when many chemicals or mixtures have to be tested simultaneously. In such cases, the simpler method that uses transfers of conidia or mycelia may actually be more laborious. If media permit fairly normal growth, abnormal (potentially aneuploid) colonies may be recognizable when conidia are plated. In addition, levels of survival can be obtained from such platings. This provides an objective measure of toxicity (useful for comparison of different studies).

(2.3.3.2) Treatment of conidia in liquid. This method is more informative, since single nuclei are treated for a short period and then grown into colonies on standard agar media. It is also more quantitative, especially if chemicals are unstable or interact with growth media. Usually, quiescent and germinating conidia will be treated.

Quescent conidia.. This method is relatively straighforward and recommended as an informative control. Most agents that induce aneuploidy seem to show effects only if dividing nuclei are exposed, while mutagens which cause secondary nondisjunction generally affect both types of nuclei. Comparison of both methods, therefore, produces useful information.

Germinating conidia. Optimal information on primary effects is obtained by treating single nuclei which are preparing to divide. In Aspergillus, conidia incubated in liquid media (at 37°C) reach that stage after 3.5–4.5 h, the exact time depending on media and strains used. Either of two basic procedures can be used to expose germinating conidia to various concentrations of the test chemical: either the chemical is added for the entire germination period until incipient germ tubes can be seen, or for a limited time (often 1 or 2 h) after a period

of pre-germination (3 or 4 h) (Table 1A). The first method generally has given more spectacular results but it can be very tedious if the length of treatment exceeds a normal working day (Shanfield and Käfer, 1971; Käfer, 1984).

In all cases, careful pilot studies using a wide range of dosages are required. Effects can easily be missed, since too low as well as too high a concentration may produce only normal survivors. High doses presumably inhibit division so that most nuclei never reach the vulnerable stage during treatment.

(2.3.4) Suggested sequence of tests

If the aim is to identify or to disprove induction of aneuploids as efficiently as possible, the following variants of the 3 methods are recommended as a sequence of tests.

(2.3.4.1) Testing for chromosome malsegregation among euploid segregants from heterozygous diploids. For this first test, conidia from well-marked diploids (**19**, or preferably **29** or **30**, Fig. 1) are exposed to the chemical during growth. Stable agents are used in agar media onto which conidia are plated; or growing colonies can be exposed to vapors in sealed vessels. When growth is very abnormal in such tests or when the test compound is unstable, quiescent and pre-germinated conidia have to be exposed in liquid and plated onto complete media. Several, somewhat inhibitory, concentrations of the test chemical are used and exposure times preferably kept constant. Colonies which are able to conidiate will show increased frequencies of color segregants if genotoxic effects have been induced. Stable yellow (*yA/yA*) segments or sectors, preferably from low-density platings of the most effective doses, are tested for linked markers to identify chromosome malsegregation or crossing-over. If effects are marginal, selective platings of the same suspensions usi..g media supplemented with PFPA can be made. These may confirm minor increases or unusual proportions of segregant types (2*n*-N will be yellow, and *n* will be beige or white, while 2*n*-CO will be green; see Section 2.2.1).

In all cases, but especially when whole colonies of segregant color are included in the analysis, or when segregants are obtained from selective media, it is essential to carry out and record simultaneous control platings. Any low-level effects can, therefore, only be judged against controls from the same conidial suspension, because mitotic recombination shows clonal distribution.

For any of these methods, it is important to record and report simultaneously the frequencies of all types of segregants, even if mitotic crossing-over is not of interest. Furthermore, if distinction is possible it is useful to record and report segregants from normal and abnormal colonies separately. Only if crossovers mainly arise as twin sectors in normal colonies and nondisjunctionals are sectors of abnormal ones, is it likely that both processes were induced independently (Section 2.1.1). If this is the case, induced aneuploidy probably can be confirmed most easily by treating haploids and identifying $n+1$ hyperhaploids (see below). If results are not that clear-cut, all colonies with *yA/yA* sectors should be analyzed further, especially those which have abnormal centers and are thought to be trisomics.

(2.3.4.2) Testing for aneuploidy among abnormals induced in diploid strains. To analyze the potential aneuploids induced, conidia from abnormal centers of individual colonies are usefully replated by needle platings to produce secondary aneuploids and a sufficient number of euploid spontaneous sectors (Käfer, 1961). Haploids among the latter will give a fair indication of the genotype of the sectoring abnormal types (large abnormal centers can be transferred to benlate media for haploidization). In this analysis, results for each individual colony have to be recorded separately, so that simultaneous production of euploid (especially *yA yA*) sectors by crossing-over and by nondisjunction from a single abnormal colony can be recognized. Conclusive evidence for or against whole-chromosome aneuploidy will be obtained by combining these data with information obtained from haploid sectors. Only if chromosomal-type diploid segregants are about one-third of all diploid sectors and if the other two-thirds are basically of a parental type is trisomy clearly indicated. Other ratios (e.g., frequent yellow nondisjunctional sectors without green diploid parental types from a green abnormal center) do not indicate aneuploidy, but a sedidominant lethal mutation in the arm of the wild-type allele for *yA* (confirmed by lack of yA^+ haploids).

Since in all diploid tester strains, only chromosome I is well-marked, analysis is usefully restricted to abnormals which produce yellow sectors. Because these may be fairly rare (no more than 1/16–1/32 among sectoring abnormals), detailed analysis of only a limited sample is usually feasible (15–30 or more). If these abnormals are aneuploids, simple $2n + 1$ trisomics with two *yA* homologues will be produced in the replatings, and typical $2n + 1$ phenotypes should be observable, as found for some of the ethanol-induced aneuploids (Käfer, 1984). Trisomics segregating for other color mutants ($2n +$ II or VIII) and trisomics with very typical phenotypes ($2n +$ III or VI) may be recognizable as well (Section 2.1.2). However, if complex aneuploids have been induced, few simple $2n + I$ types may be observed among the many types of sectoring colonies. Repeated replating of such abnormals will eventually produce simple, recognizable trisomics, but this procedure is slow and not suitable for routine tests.

As an alternative, abnormals can be analyzed by streaking their centers on complete media and testing yellow sectors from individual streaks. In this procedure the correlation of genetic results with characteristic phenotypes is lost, and the ratios among different sectors which are typical for trisomics are not recognizable.

(2.3.4.3) Visual identification of aneuploids from treated haploids. When diploid conidia produce a wide variety of apparent aneuploids, treatment of haploids is recommended as an alternative simpler test. Fewer types but also fewer surviving cases are expected when malsegregation is induced in haploids. True aneuploids on replating will show frequent sectors and produce some of the standard recognizable $n + 1$ types (Table 5). Alternatively, stable abnormals, presumable mutant types, may be found (e.g., after treatment of quiescent conidia with MMS or ionizing radiation). In general, these tests are very clear-cut and conclusive, but classification is not always unambiguous. A wider variety of types is obtained after treatment with agents which also induce polyploidy (e.g., diploids and hyperdiploids, as found for chloral hydrate; Table 4; Käfer, 1985). In these latter cases, evidence for diploid segregants provides a clear indication of primary effects on chromosome segregation (Section 2.1.3).

(2.3.5) Positive and negative controls; vehicles and solvents

A special problem with tests for induced nondisjuction arises from the fact that effects are restricted to dividing nuclei. For quantitative analysis, germinating, but still mononucleate, conidia must be treated with increasing concentrations of a chemical. High concentrations may block division and reduce induction of aneuploidy. Therefore, cells have to be treated with a narrow range of concentrations in which division is affected but not completely blocked. Consequently, it may be very difficult to demonstrate dosage effects. When only one or very few dose levels show effects, simultaneous positive, and sufficiently extensive negative, controls become very important in demonstrating significance of small increases. Positive controls are an important check on procedures when preincubation is used (e.g., ruling out treatment of multinucleate stages).

Benlate or PFPA are most useful as positive controls. They produce many haploids, permitting verification of the genotype of the test diploids as part of the analysis of positive control samples (Section 2.2.3). In addition, treatment with a clastogen which induces abnormals that are not aneuploids, should be added for comparison.

For negative controls solvents should be used. This is all the more important, because some frequently used ones induce malsegregation at concentrations which are barely higher than those commonly used (e.g., ethanol, known to induce segregation at concentrations of $< 3\%$ in dividing cells; Käfer, 1984). Furthermore, deoxycholate is routinely used at a concentration (0.8 g/l) which causes considerable kill and probably induces aneuploids (Table 2; Assinder and Upshall, 1982). Enhancing effects of these chemicals, especially if used in combination with PFPA or primaricin, need to be ruled out in control samples of sufficient size.

(3) Interpretation of data

Results for all chemicals for which acceptable test data were reported are classified in Table 6; chemicals are listed alphabetically. Summaries of the original published data of papers that were published by 1979 have been presented in detail

and discussed in the previous review of tests for genotoxic effects on mitotic recombination in Aspergillus (Tables 1–4 and 6 of Käfer et al., 1982).The specific tables of this earlier review are referred to in the second-last column of Table 6 (as t1–t4 and t6, to distinguish these from Tables 1–5, i.e., T1–T5, of the current review). Tests for which no raw data had been published were excluded from current considerations.

TABLE 1

EFFECT OF VARIOUS CHEMICALS ON FREQUENCY OF EUPLOID SEGREGANTS, RESULTING FROM CHROMOSOME MALSEGREGATION (2*n*-N AND *n*) OR FROM CROSSING-OVER (2*n*-CO) IN COLONIES GROWN FROM CONIDIA AFTER 'LIQUID EXPOSURE'

A. Effects of pre-germination before treatment

Chemical: Concentration (mM) (hours of treatment)	Effects on conidia						Diploid No. [a] (Fig. 1) and reference
	Quiescent			Pre-germinated (3 or 4 h)			
	Survival (%)	Segregants (per 1000)		Survival (%)	Segregants (per 1000)		
		2*n*-N and *n*	2*n*-CO		2*n*-N and *n*	2*n*-CO	
MMS (1 h)							
0 [b]	100	0.6	2	100	0.6	2	**4** [a]
4.5	100	7	2	100	6	12	Gualandi et al., 1979
9.0	55	20	16	20	17	20	
11.4	–	–	–	2	25	120	
18.2	9	85	50 [c]	–	–	–	
0 [c]	100		0.2	100		0.1	
4.5	50	NS	0.3	100	NS	0.5	
11.4	25		0.4	20		0.5	
18.2	10		0.5	–		–	
0 [d]	100	0.01	0.04	100	0.03	0.02	**23** [d]
1.8	93	0.02	0.1	93	0.1	0.5	de Bertoldi
7.2	97	0.02	0.5	7	0.2	8.7	et al., 1980
9.0	44	0.05	0.9	1.5	0.3	12.1	
Benomyl (1 h) [d]							
0	NED [e]			100	0.06	0.03	**23** [d]
0.35				96	0.4	0.03	de Bertoldi
1.7				81	1.0	0.03	et al., 1980
2.8				69	2.7	0.05	
p-Fluorophenylalanine (1 h) [d]							
0	NED			100	0.04	0.02	**23** [d]
0.5				63	0.25	0.02	de Bertoldi
2.0				48	0.9	0.04	et al., 1980
4.0				33	1.8	0.02	
CIPC [f] (2 h) [b]							
0	NED			100	1	NS	**4** [a]
0.2				100	5		Gualandi and
0.4				9	60		Bellincampi, 1981
Mitomycin C (2 h) [b]							
0	NED			100	1	NS	**4** [a]
1.2				100	5		Gualandi and
3.0				85	5.5		Bellincampi, 1981

TABLE 1 (continued)
B. Effects of testing method and metabolic activation

Chemical: Concentration (and period of treatment)	Effect of conidia under different test conditions						Diploid No. [a] (Fig. 1) and reference
	Survival (%)	Segregants (per 1000)		Survival (%)	Segregants (per 1000)		
		2*n*-N and *n*	2*n*-CO		2*n*-N and *n*	2*n*-CO	
MMS (mM) (> 3 days)	Agar incorporation ('plate test')						
	Selective method (*fpaA*) [c]			Nonselective (*yA*) [b]			4 [a]
0	100	0.2	0.2	100	0.7	2	Gualandi et al., 1979
0.23	100	NED	15	100	NED	120	
0.45	100		120	80		250	
0.91	65		100	35		140	
1.82	10		12	–	–	–	
Bleomycin (μM) (7 days)				Nonselective (*yA*, *wA*) [b]			**16** [a]
0				100	200	450	Demopoulos
0.07				94	260	1620	et al., 1982
0.10				93	240	1960	
0.14				88	270	2630	
0.35				85	230	2620	
Trifluralin [g] (mM)	'Spot test' [g] Selection for *fpaA* [c]			Liquid exposure of germinating conidia (single experiment)			**4** [a]
0	NS [e]	NED	2.6 ± 0.7	NS	2.0	NS	Carere and
0.3			14.3 ± 5.7		–		Morpurgo, 1981
1.5			12.0 ± 1.2		5.5		
3.0			10.0 ± 1.6		10.6		
14.9			17.3 ± 3.5		2.2		
29.8			–		3.4		
Cyclophosphamide [d] (mM) (4 h, 37°C) [h]	Liquid exposure of conidia [h] and metabolic activation						
	Without activation			With activation			**23** [d]
+0	100	0.04 ± 0.01	0.05 ± 0.03	100	0.2 ± 0.02	0.3 ± 0.1	de Bertoldi
4	31	0.05 ± 0.02	0.09 ± 0.02	30	0.9 ± 0.03	0.8 ± 0.1	et al., 1980
8	29	0.05 ± 0.01	0..10 ± 0.02	6	2.9 ± 0.08	3.1 ± 0.1	
16	30	0.16 ± 0.03	0.09 ± 0.02	0.4	15.8 ± 0.94	9.8 ± 0.5	

[a] See Käfer et al. (1982), Fig. 1 for diploids Nos. **1–7**, Fig. 2 for diploids **8–20**.
[b] Nonselective method; yellow sectors tested for *pabaA*, and white sectors for *thiA* and *cnxE*, to distinguish 2*n*-N from 2*n*-CO.
[c] Selection for *fpaA* on supplemented MM after 'liquid exposure' (haploids unable to grow); for results in 'plate test' see Table 1B.
[d] Selection for *pimA* (resistance to pimaricin) produces green 2*n*-CO segregants and yellow 2*n*-N and *n*, the latter in ratios of 1 : 24 (in controls and treated samples).
[e] NED, no effect detected; NS, not stated.
[f] CIPC, isopropyl-3-chlorophenylcarbonate (pesticide); all segregants haploid.
[g] Related trifluorotoluene derivatives (CTT, NCTT, DNCTT) showed no effects in equivalent tests.
[h] Conidia when plated after 4 h at 37°C show unknown amounts of germination, dependent on the inhibitory effects at different dose levels.

(3.1) Presentation of data

Recent papers (1979–1984) that contained acceptable results are summarized in Tables 1–4 in the current review. As far as possible, each table contains data which were obtained by comparable test systems and by similar methods of exposure.

Table 5 shows details on identified aneuploid types, as far as available from some of the tests reported in Tables 2–4.

(3.1.1) Specific features of summarized results

Table 1A summarizes data from tests which do not identify aneuploids but measure increases in the frequencies of stable (euploid) segregants in colonies grown from quiescent and pre-germinated diploid conidia which were treated in liquid. These include tests of well-known haploidizing agents and mutagens. The former generally were used as positive controls (e.g., PFPA and benomyl, for validation of the *pimA*/+ selective system by de Bertoldi et al., 1980). Typically in such cases, increases in frequencies of malsegregants ($2n$-N and n types) were observed only when pre-germinated conidia were treated.

On the other hand, mutagens, e.g. MMS, increased all types of segregants, in quiescent and in germinating conidia. This effect was found for MMS only in 1 of 3 test systems, namely the nonselective tests of Gualandi et al. (1979; Table 1A). In contrast, selective tests, or tests in which MMS was incorporated into growth media (Table 1B) demonstrated mainly increases in crossing-over. This clearly shows that either no malsegregants were induced by MMS or that such segregants cannot be reliably detected by the latter tests which, however, are able to detect induction of haploids by PFPA or benomyl. Therefore, it is not clear whether MMS induces 'nondisjunction' as postulated by Gualandi et al. (1979). The mutagenic effects of MMS may have led to secondary malsegregation under favorable circumstances, but some primary effect is not ruled out and more conclusive tests are needed. Whether the same applies to trifluralin (Table 1B) is hard to judge. The results reported for the two types of segregation are based on different tests, and evidence for malsegregation comes only from preliminary data. Similarly, the results reported for mitomycin C and CIPC (Gualandi and Bellincampi, 1981; Table 1A) indicate some effects on malsegregation but are too limited to judge whether these are primary or secondary.

The effects of cyclophosphamide, which is known to be mutagenic, are well-documented and interesting (de Bertoldi et al., 1980; Table 1B). This chemical showed very high increases of all types of segregants but only when it was metabolically activated and incubated for 4 h (during this time it is likely that germination may have occurred). As in the preceding cases, the reported results suggest occurrence of malsegregation as a secondary process.

The tests summarized in Tables 2–4 show very clear primary induction of aneuploidy, at least in all those cases in which aneuploids were actually identified: ethyl alcohol, chloral hydrate, Thiram and sodium deoxycholate (Table 5). Conclusions for the other cases are based on the finding of abnormal colonies with sectors which result exclusively from whole-chromosome segregation. The chemicals involved are various membrane-damaging agents (Bellincampi et al., 1980, Tables 2 and 3) and are listed as primary inducers (i.e. '1') in Table 6 (on the assumption that crossing-over, which is not reported, showed normal frequencies).

The results from treatment of haploids are shown in Table 4, but only tests which have been fully analyzed (Table 5) are included. An incomplete analysis of unstable, possibly aneuploid, abnormals, obtained after treatment of haploids with acriflavine by Ball and Roper (1966), is omitted from Tables 4 and 6.

(3.1.2) Problems with dose–response curves

As mentioned above (Section 2.3.5), inducers of primary malsegregation show poor dose–response relationships when aneuploid frequencies are determined (Tables 2 and 3). At very high concentrations, where division of nuclei does not progress to the 'sensitive stage' within the alloted treatment period, all survivors may be normal (e.g., at concentrations of over 7% ethanol). Similarly, if treatment periods are kept constant and concentrations are uniformly increased, aneuploid frequencies do not increase in parallel because different stages of division may have been exposed. An impractical solution is to treat conidia at each concentration until germination starts. Because of poor synchrony such periods are not the same for all spores and results are highly variable (Table 3, results for ethanol).

Within a narrow range, at low kill, consistent increases may be obtained (e.g., after treatment of pre-germinated conidia with PFPA or benomyl;

Table 1A; de Bertoldi et al., 1980). A partial exception seems to be the finding for deoxycholate (Assinder and Upshall, 1982; Table 2): when conidia were plated onto media containing a wide range of concentrations, consistent (but not parallel) increases of aneuploids were obtained down to 1% survival.

TABLE 2

FREQUENCIES OF ANEUPLOIDS FROM DIPLOID CONIDIA PLATED ONTO COMPLETE MEDIUM + CHEMICALS

Aneuploids identified by exclusively chromosomal-type segregation of markers in euploid sectors and/or phenotype of abnormal colonies

Chemical in CM during growth (incubation temperature)	Concentration (mM)	Toxicity Survival (%)	Frequency of abnormals (% ± S.E. or	sample size) (%)	Abnormals segregating for chromosome I	Diploid No. [a] (Fig. 1) and reference
Historical control	0	100	0.3	3374	0.8	**4** [a]
Control	0	100	0	479	0	Bellincampi et al., 1980
Miconazole	0.001	70.8	3.1	510	1.2	
	0.01	3.0	23.1	350	17.1	
Econazole	0.0007	2.0	30.0	50	16.0	
	0.0013	0.5	68.0	57	61.0	
Control	0	100	0.05	2244		**22** Upshall and Johnson, 1981
Thiram	0.08	25	22	723	ND [b]	
	0.10	31	29	687		
	0.12	30	42	662		
	0.15	17	39	396		
	0.17	13	39	310		
Deoxycholate (37°C)	0.7	100	2		ND [b]	**22** Assinder and Upshall, 1982
	1.5	60	3			
	2.2	40	5			
	3.0	20	7	350		
	3.7	2–3	10			
				to		
(25–30°C)	0.7	90	2			
	1.5	30–40	5–8	700		
	2.2	5–10	12–15			
	3.0	2	35–40			
	3.7	1	60			
	% (v/v)					
Control	0	100	0.6 ± 0.1			**19** [a] Käfer, 1984
Ethanol	2.5	81 ± 8	5.5 ± 1.5		ND [b]	
	4–5	37 ± 6	23.5 ± 4			
	6.5	35 ± 1	18.1 ± 2			
	7.5	11.5 ± 2	13.5 ± 1.5			

[a] For diploid Nos. **1–20** see Figs. 1 and 2 of Käfer et al. (1982).

[b] ND, not determined; for details of analyzed samples, see Table 5.

(3.1.3) Data transformation

The lack of a consistent dose relationship precludes the type of transformation of raw data that is used for tests of mutagenicity, based on the excellent correlation between survival and mutation frequency (Scott et al., 1982). At this point the most informative procedure will be to agree on some standard protocol for production of quantitative, comparative data (e.g., treatment of conidia in liquid for 2 or 1 h, after pre-germination for a period of 3 or 3.5 h, respectively). The effective dose range of each chemical under these condi-

TABLE 3

FREQUENCIES OF UNSTABLE ABNORMAL COLONIES OBSERVED AMONG SURVIVORS OF DIPLOID CONIDIA TREATED DURING GERMINATION OR AFTER PRE-GERMINATION [a]

Chemical and concentration	Pre-germination (h)	Treatment (h)	Toxicity Survival (%)	Abnormals %	Abnormals Total	Abnormals [b] with malsegregation for chromosome I (%)	Diploid No. (Fig. 1) and reference
Ethanol (%) (v/v)							
0	3+1	0	100	0	0	0	**26**
3	3	4	40	4	190	ND [c]	Käfer, 1984
		5–6	16	17	148		
4	3	3–4	33	10	243		
		5–6	30	5	291		
5	3	3–4	35	5	254		
		5–6	19	9	140		
0	4	0	60	1	NS [c]	0	
4	0	12–13.5	30–82	31		8	
		14–15.5	34–76	42			
4.5	0	13.5–15.5	65–75	45		12	
5	0	13.5–15.5	23–64	62		10	
		12–13	41–60	10			
		14	31	7			
Amphotericin B (μM)							
0 = control	4	0	100	0.2 [b]	489	0.2	**4** [d] Bellincampi et al., 1980
0.55	4	2	74.0	1.9	351	0.6	Bellin-
1.08	4	2	9.2	6.8	190	3.7	campi-
2.16	4	2	1.9	17.0	387	5.7	et al., 1980
Pimaricin (μM)							
0 = control	4	0	100	0	1184	0	
14.4	4	2	44.9	1.2	423	1.2	
21.6	4	2	44.7	1.4	504	0.7	
28.8	4	2	6.8	1.1	660	1.4	
43.1	4	2	1.7	13.6	293	3.7	
Fenarimol (μM)							
0 = control	4	0	100	0.1	705	0	
	0	7	15.0	5.5	162	4.3	
	0	7	6.0	10.8	268	9.7	

[a] No effect on quiescent conidia (unpublished results).

[b] Abnormals concluded to be aneuploids; similar information for other chromosomes indicates random malsegregation.

[c] ND, not determined in detail, NS, not stated.

[d] See Fig. 1, Käfer et al. (1982).

tions can be determined. When quantitative data have been accumulated within identified narrow dose ranges, results can be compared with appropriate mathematical models.

The situation is probably different for secondary nondisjunction, where frequencies are expected to show dose relationships similar to those of mutation if the primary cause is a mutagenic type of DNA damage. When sufficient data are available, data transformation of the type proposed by Munson and Goodhead (1977) will be useful as outlined in detail by Scott et al. (1982).

(3.1.4) Units of expression

These units change and differ between simple tests of segregants in diploids and more complex tests which identify aneuploids, from frequencies of euploid malsegregants per survivor or colony to frequencies of sectoring abnormal (putative aneuploid) types among total number of colonies. It is evident from the discussions above that further crucial information is obtained from the relative frequencies of all segregant types. These are, in the case of euploid segregants, the ratios of malsegregants to crossover types and, among malsegregants ($2n$-N and n types), the proportion of haploids (especially if these differ from controls). Among abnormal colonies it is imperative to identify the ratio of genuine aneuploids to other types which may also show sectors by (secondary) malsegregation of chromosomes.

TABLE 4

FREQUENCIES OF HYPERHAPLOID AND DIPLOID COLONIES, INDUCED BY TREATMENT OF HAPLOID GERMINATING CONIDIA WITH ETHANOL OR CHLORAL HYDRATE [a]

Concentration	Pre-germination (h)	Treatment (h)	Survival (%)	Types with altered ploidy		
				Aneuploids		Diploids [b] (%)
				% ± S.E.	Sample size	
Ethanol (%) (v/v)						
0	3.5 + 1	0	(61 ± 16) [c]	0.7 ± 0.4		
3	3.5	1–4	23 ± 2.5 [d]	1.8 ± 1.0		ND [e]
4	3.5	4	24 ± 4	4.0 ± 2.0		
		6	43 ± 5	4.3 ± 1.1		
5	3.5	4	37 ± 9	4.0 ± 2.0		
	3.5	5	16 ± 4	3.5 ± 1.4		
0	3 + 1.5	0	(< 100) [c]	2	57	
3	3	3–4	22 [d]	2.5	144	
		5–6	15	6	171	
4	3	3–4	17	8	150	
		5–6	27	3	240	
5	3	3–4	30	2	273	
		5	11	7	59	
		6	27	2	86	
Chloral hydrate (mM)						
0	3 + 1.5	0	(55) [c]	1.5	672	0
10	3	3–5	24 [d]	24	271	58
		6	7	43	369	34
15	3	5	11	15	320	66
		6	7	22	147	47
20	3	6	18	14	393	69

[a] Strain *anA biA* (Käfer, 1984, 1985).
[b] Visual identification.
[c] Compared to quiescent control.
[d] Percentage of germinating control.
[e] ND, not determined.

TABLE 5

RELATIVE FREQUENCIES OF ANEUPLOID TYPES IDENTIFIED IN SAMPLES OF REPLATED ABNORMALS INDUCED BY VARIOUS CHEMICALS AS SUMMARIZED IN TABLES 2–4

Ploidy of treated strain and chemical	Relative frequencies of aneuploid types						Sample size	Reference
	Hyperdiploid only		Hyperdiploid or segregant $2n$ and hyperhaploid (%)	Hyperhaploid only				
	$2n+1$ or $2n+2$ (%)	$2n+3$ or more (%)		$n+1$ (%)	$n+2$ (%)	$n+3$ or more (%)		
Diploid treated [a]								
Ethanol	37	3	39	9	5	6	78	Käfer, 1984
	36	23	37	1	1	2	173	
Thiram	42		42	16			24	Upshall and Johnson, 1981
Deoxycholate	6	12	22	38	12	12	50	Assinder and Upshall, 1982
Haploid treated [b]								
Ethanol	3	?	2	68	21	6	95	Käfer, 1984
Chloral hydrate	42	2	4	26	15	6	102	Käfer, 1985

[a] 15–100 euploid sectors from each abnormal tested for segregation of markers.

[b] Visual identfication of aneuploids; number of extra chromosomes deduced from number of $n+1$ types among replated aneuploid conidia.

(3.2) Criteria for acceptability of data

The following criteria have been established for acceptability of results (Scott et al., 1982) and were applied here (with minor relaxations).

(3.2.1) Adequate controls

At least one measurement of control frequencies, simultaneously obtained among solvent-treated conidia, is required. Information on the variability of the historical control is needed in addition, when the significance of small effects is at issue. This was not a problem in the present review because, in general, only drastic positive results had been published in detail. The dearth of published negative results should be remedied.

(3.2.2) Adequate documentation, including raw data

Results were included if raw data (actual numbers, or frequencies and approximate size of samples) were reported.

(3.2.3) Dose response and / or adequate sample size

Results were classified as positive when observed increases were statistically significant or several values clearly increased with dose. In addition, cases were included when at least two obviously effective dose values were reported or single values showed increases of at least 5-fold.

(3.3) Statistical evaluation

As discussed above (Sections 3.1.2–3.1.4) no adequate statistical treatment has been identified for frequencies of primary aneuploids, which often are not measured in sufficient detail within the narrow range where effects increase with dose. Current efforts have concentrated on identification of agents that produce drastic effects (often 10–100-fold increases), which are obviously significant (chi-square tests can be used to check for significance, provided no expected value is less than 1 and no more than 20% of the expected values are between 1 and 5 (Cochran, 1954; Roscoe and Byars, 1971)).

For cases of mutagens which indirectly cause secondary malsegregation and do not induce primary aneuploidy, dose relationships are expected to be suitable for linear regression analysis (except if kill is very high, with survival of less than 2% (e.g., de Bertoldi et al., 1980)). For com-

parison of results with different chemicals, the transformation, presentation and analysis of data according to the model of Munson and Goodhead (1977) should be applicable (Scott et al., 1982).

(3.4) Criteria for positive / negative / inconclusive results

In the reviewed publications, practically only positive results have been reported as raw data, or in sufficient detail, to be included and almost all showed large increases in whole-chromosome malsegregation (listed as '+' in Table 6). Negative results were well-documented almost exclusively in cases which showed increases in crossover segregants and for this reason were reported in detail (e.g, results for bleomycin; Demopoulos et al., 1982).

In addition to criteria for sufficient documentation (Section 3.2), the main concern in the classification of results arises from the narrow scope of this review, which is meant to exclude clastogens and to identify agents that induce whole-chromosome aneuploidy. This limitation presents a problem, because the simplest test systems in Aspergillus only identified euploid genetic segregants, some of which are postulated to be indicative of malsegregation of whole chromosomes. Such segregants can, however, originate equally well from aneuploidy of whole or of partical chromosomes. The task, therefore, was to decide on broad criteria which would apply to both types and on more specific criteria that would distinguish between them. In Table 6 all chemicals are listed as positive if they caused significant increases of haploid or diploid whole-chromosome segregants. However, if induced aneuploidy was actually demonstrated, the chemical most likely caused malsegregation as a primary effect ('1' in Table 6). On the other hand, chemicals which were shown to increase the frequencies of mutants and/or crossovers in addition to those of malsegregants were classified as causing secondary malsegregation ('2' in Table 6). This latter classification will not be correct for any agent that can induce both primary aneuploidy and crossing-over or mutation. Such a case may be the mutagen MNNG which clearly induced reciprocal crossing-over as well as aneuploidy (and possibly MMS). Similarly, benlate which induces aneuploidy apparently is able to increase mutation in Aspergillus, but only in repair-deficient strains (Kappas and Bridges, 1981).

Lack of information is indicated as '1 or 2' in Table 6 and includes mainly cases which need further analysis, especially testing for aneuploid primary products. One of two cases classified as inconclusive is mitomycin C (Gualandi and Bellincampi, 1981; Table 1A); it showed no dose effect but a 5-fold increase, and no raw data were published. The other case is FUDR, for which classification was also somewhat ambiguous since it showed predominantly induced crossing-over, but in addition caused a slight increase in frequencies of haploids or 'abnormals' (Shanfield and Käfer, 1971). In such cases the question arises, whether it is legitimate to classify segregants as resulting from malsegregation when heterozygous markers on both arms of a chromosome are found to be homozygous. Arguments based on spontaneous frequencies do not hold; when crossing-over is greatly increased, double crossovers are not expected to be 'much rarer' than nondisjunctional diploid segregants, but actually may be more frequent (sulfanilamide is, therefore, listed as 'negative'; Käfer et al., 1976). A useful criterion to distinguish the two types of segregants is the differential in growth rate. An increased rate is not expected for double crossovers but is found for nondisjunctional diploid segregants because these generally originate in trisomics. Observation of phenotypes of colonies, therefore, adds the equivalent of cytology to this assay and should be used consistently. (There is a minor problem of terminology, since the term 'sectoring colonies' is used to identify 3 different observations. In some papers this term refers to diploid colonies with euploid colored segments or patches; in others, it specifies abnormal, including aneuploid, colonies with better growing, often euploid, sectors. In a third group of papers, it covers both types or, worse, it means the latter but does not state this clearly.)

Based on these criteria, about half of the chemicals listed in Table 6 (22 cases) have either been shown to cause primary aneuploidy (12 cases) or are very likely able to cause such effects (10 cases). The former group includes well-known

TABLE 6

CHEMICALS TESTED IN ASPERGILLUS FOR EUPLOID SEGREGANTS RESULTING FROM MALSEGREGATION (ASSAY CODE 1) OR FOR ANEUPLOIDY (ASSAY CODE 2) AND CLASSIFICATION OF INDUCED EFFECTS AS EITHER PRIMARY MALSEGREGATION (TYPE 1) OR AS INDUCED DNA DAMAGE AND SECONDARY MALSEGREGATION (TYPE 2)

+, positive; (+), weakly positive; I, inconclusive evidence; −, negative response.

Chemical	CAS No.	Results						Assay code [b]	Table in reviews [c]	References
		Summary		Doses tested		LEDT or HIDT [a]				
		+ or −	Type 1 or 2	Concentration (μM) (or time)	(No.)	Concentration (μM)	Time (days or hours) [a]		t 1982 T 1985	
Actinomycin D	50-76-0	+	2?	10–20	(3)	10	7 d	2b	t1	Kappas, 1978
Amitrole	61-82-5	(+)?	1 or 2	Range, 4757 max.	(NS)	4757	3–4 d	1a, 1c	t2	Bignami et al., 1977
Amphotericin B	1397-89-3	+	1	0.5–2.2	(3)	1.1	2 h	2b	T3	Bellincampi et al., 1980
Benomyl	17804-35-2	+	1	0.7–1.55	(3)	0.7	3–4 d	2b	t2	Bignami et al., 1977
				0.5–1.5	(3)	0.5	7 d	2b	t1	Kappas, 1978
				(1–4 h)	(4)	2.7	1 h	1a	t4	Morpurgo et al., 1979
				350–2800	(4)	350	1 h	1c	T1A	de Bertoldi et al., 1980
Bleomycin	11056-06-7	−	NA	0.07–0.35	(4)	0.35	7 d	1a	Text	Demopoulos et al., 1982
Botran	99-30-9	+	2	10–30	(3)	10	7 d	2b	t1	Kappas, 1978
Captan	133-06-2	−	NA	Range, 40 ppm max.	(NS)	40 ppm	3–4 d	1a	t2	Bignami et al. 1977
Carboxin (Oxathiin or Vitavax)	5234-68-4	(+)	1 or 2	42.5	(1)	42.5	2 d	1b	t1	Azevedo et al., 1977
Chloral hydrate	302-17-0	+	1	1000–40000	(4)	20000	5 d	1a	t3	Singh and Sinha, 1976, 1979
				10000–20000 (3.5–6 h)	(5)	10000	3.5 h	2a	T4, T5	Käfer, 1985
Chlorfurazol (DTFB)	3615-21-2	−	NA	12–30	(3)	30	7 d	1b	t1	Kappas et al., 1974
Chloroneb	2675-77-6	+	2(?)	48.3	(1)	48.3	4 d	1b	t1	Azevedo et al., 1977
				20–40	(3)	20	7 d	2b	t1	Kappas, 1978
Chlorpropham (CIPC)	101-21-3	+	1 or 2	200–400	(2)	200	2 h	1a	T1A	Gualandi and Bellincampi, 1981
Cyclophosphamide	50-18-0	−	NA	4000–16000	(3)	16000	4 h	1c	T1B	de Bertoldi et al., 1980
+ 'S9' activation		+	2	4000–16000	(3)	4000	4 h			
Dichlorvos	62-73-7	+	2	Range, 3620 max.	(NS)	3620	3–4 d	1a	t2	Bignami et al., 1977
Diepoxybutane (DEB)	1464-53-5	+	2	(0.5–1 h)	(2)	100000	0.5 h	1a	t4	Putrament, 1967
Dimethyl sulfoxide	67-68-5	−	NA	Wide range, 52475 max.	(NS)	52475	3–4 d	1c	t2	Bignami et al., 1977
							3–4 d	1a	t3 [d]	Morpurgo et al., 1979
Econazole	27220-47-9	+	1	0.65–1.31	(2)	0.65	2–3 d	2b	T2	Bellincampi et al. 1980
Ethanol	64-17-5	+	1	0.5–5%	(3)	5%	3 d	2b	t6	Harsanyi et al., 1977
				6–8%	(3)	6%	NS	1a	t4	Morpurgo et al., 1979
				3–7.5% (3 h–3 d)	(40)	3% = 615000	5 h	2a	T2–T5	Käfer, 1984
Ethidium bromide	1239-45-8	−	NA	3.8–5.1	(2)	5.1	7–8 d	1b	t1	Bonatelli and Azevedo, 1977
Fenarimol	60168-88-9	+	1	18.1–36.2	(2)	18.1	7 h	2b	T3	Bellincampi et al., 1980
5-Fluorodeoxyuridine (FUDR)	50-91-9	I	NA	406 (12 h)	(1)	NA	12 h	1a	t6	Shanfield and Käfer, 1971

Griseofulvin	126-07-8	+	1?	11.3–70.8	(5)	11.3	5 d	1b	t1	Kappas and Georgopoulos, 1974
Iodochloro-hydroxyquinoline	130-26-7	+	1?	(0.5–6 h) [c]	(5)	Gradient saturated → 0 [c]	3 d	1c	Text, 1982	Käfer et al., 1976 Bignami et al., 1974
Mecoprop	93-65-2	–	NA	1631–1864	(2)	1864	3–4 d	1a	t2	Bignami et al., 1977
2,3-Methoxycarbonyl-thioureidoaniline	27079-29-4	+	1 or 2	2–7	(3)	2	7 d	1b	t1	Kappas et al., 1974
MBC (methyl 2-benz-imidazolecarbamate)	10605-21-7	+	1 or 2	1–2.5	(3)	1	7 d	1b	t1	Kappas et al., 1974
Methyl methane-sulfonate (MMS)	66-27-3	+	2?	4540–11 350	(3)	4540	1 h	1a	T1A, B	Gualandi et al., 1979
				1800–9000	(4)	1800	1 h	1c	T1A	de Bertoldi et al., 1980
Methyl thiophanate	23564-05-8	+	1 or 2	9–16	(3)	9	7 d	1b	t1	Kappas et al., 1974
Miconazole	22916-47-8	+	1	1–10	(2)	10	2–3 d	2b	T2	Bellincampi et al., 1980
Mitomycin C (MMC)	50-07-7	!	(2)	1200–3000	(2)	NA	2 h	1a	T1A	Gualandi and Bellincampi, 1981
Natamycin (pimaricin)	7681-93-8	+	1	16–80	(4)	16	2 h	2b	T3	Bellincampi et al., 1980
N-Methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG)	70-25-7	(+)	1?	[4 strains]	(1)	3400	0.05 h	2a	t6	Shanfield and Käfer, 1971
4-Nitroquinoline *N*-oxide (4NQO)	56-57-5	+	2?	(0.25–1 h)	(3)	2.6	0.25 h	1a	t4	Morpurgo et al. 1979
p-Fluorophenyl-alanine (PFPA)	60-17-3	+	1	20000–40000	(2)	20000	10 h	2a	t6	Shanfield and Käfer, 1971
				500–4000	(4)	1000	1 h	1c	T1A	de Bertoldi et al., 1980
Picloram	1918-02-1	–	NA	Range, 3315 max.	(NS)	3315	3–4 d	1a	t2	Bignami et al., 1977
Sodium deoxy-cholate (SD) 37°C	302-95-4	(+)		772–3859	(5)	2315	2 d	2a	T2, T5	Assinder and Upshall, 1982
		(+)	1	772–3859	(5)	1544	2 d			
Sulfanilamide	63-74-1	–	NA	Range, 290 max. (0.5–6 h) [c]	(NS)	290	3–4 d	1b	t3	Morpurgo et al., 1979
					(5)	Gradient satur. → 0 [c]	3 d	1c	Text, 1982	Bignami et al., 1974 Käfer et al., 1976
		(+)?	2							
Thiabendazole	148-79-8	+	1 or 2	5–15	(4)	5	7 d	1b	t1	Kappas et al., 1974
Thiophanate	23564-06-9	+	1 or 2	8–16	(3)	8	7 d	1b	t1	Kappas et al., 1974
Thiram	137-26-8	+	1	83–166	(5)	83	4 d	2a	T2, T5	Upshall and Johnson, 1981
TTFB (4,5,6-tri-chloro-2-trifluoro-methylbenzimidazole	2338-27-4	–	NA	10–28	(3)	28	7 d	1b	t1	Kappas et al., 1974
Trifluralin	1582-09-8	(+)?	2?	1491–2982	(4)	2982	2 h	1a, 1c	T1B	Carere and Morpurgo, 1981

[a] LEDT, lowest effective dose tested; HIDT, highest ineffective dose tested; exposure time in days (d) indicates 'agar incorporation' of chemical (growing mycelium treated); exposure time in hours (h) indicates treatment in 'liquid' (usually pre-germinated conidia).

[b] For explanation of subcategories of assay codes, see text, Section 2.1.4.

[c] T1–T5 refers to tables in present review; t1–t6 to tables in preceding review (Käfer et al., 1982).

[d] Erroneously reported as + in Käfer et al. (1982, Table 3).

[e] Spot tests: triangle of filterpaper with saturated solution put for various times on top of agar media which contain conidia.

haploidizing agents, some of which have been shown to affect spindle formation, function or components (e.g., MBC, benlate and PFPA, Davidse and Flach, 1977; Morris and Oakley, 1979; griseofulvin, Crackower, 1972; chloral hydrate, Mercer and Morris, 1975). It also includes agents which were tested because of their known effects on membranes (Table 1 and 2; tests of Bellincampi et al., 1980). Among those cases for which evidence is incomplete, some chemicals are listed which may well share the active compound with benlate, namely several benzimidazole fungicides, especially MBC (Kappas et al., 1974). Not all of these are active, however, exceptions being chlorfurazol (DTFB) and TTFB. An interesting job for the future is to compare chemical composition with biological effectiveness and identify crucial molecular structures, as attempted for mutagenicity of dichlorvos and other pesticides by Morpurgo et al. (1977). Eventually such knowledge and understanding will help to predict effects of new compounds.

Of special interest are also the results for ethanol. Because of its general importance, extensive analysis was carried out to identify primary aneuploids and to rule out other effects which remained a possibility even after the detailed analysis of Harsanyi et al. (1977) and confirming results by Morpurgo et al. (1979). There is no doubt that in vegetative cells of Aspergillus, alcohol causes extensive primary malsegregation (Käfer, 1984). Related observations in early stages of developing mouse embryos (Kaufman, 1983) may extend this finding to mammalian cells, even though the effect of alcohol on abortions has not been conclusively linked to aneuploidy (Harlap and Shiono, 1980).

(3.5) Applicability of results to hazard evaluation

Since very little is known about differences between meiosis and mitosis in the function of the division apparatus (e.g., the spindle), it is uncertain at this time how informative mitotic tests are for the human meiotic situation. There is a possibility that Aspergillus tests are more (or mainly?) useful for evaluation of effects on somatic cells. Tests with diploid strains of Aspergillus share a number of features with tests using mammalian cells in culture (e.g., delayed aneuploidy presumably due to secondary segregation and competition during growth). It is not clear at this point whether the fact that fungal chromosomes show somatic pairing causes a fundamental difference for such secondary events. Tests in Aspergillus might actually share additional features with premeiotic and meiotic cells. Certainly, effects of heterozygous aberrations which cause secondary nondisjunction are well-known in both. These considerations would, of course, apply equally to somatic tests in Drosophila (Würgler et al., 1983) or mitotic tests in yeast (Esposito et al., 1982).

Correlation of results between these various systems is difficult, not only because few chemicals have been tested in all of them, but also because each system provides different secondary information on possible multi-step events in the dividing cells. If properly exploited, the Aspergillus system is probably one of the most informative at present. Only extensive comparisons in the future, especially with meiotic mammalian data, will permit judgement of its applicability to the hazard of aneuploidy in man. In this respect, it also has to be remembered that fungi have intranuclear mitotic spindles and presumably differ at least in a few molecular aspects from higher organisms.

(4) Conclusions

(4.1) Strength and weaknesses of the assay

An advantage of the assay with Aspergillus diploids is the finding that information on a wide variety of genotoxic effects is obtained simultaneously. Furthermore, several different procedures can be used to follow up on any aspect that is of special interest. However, none of the procedures which identify aneuploids are as simple as the 'Ames test', and for conclusive analysis a fairly large number of colonies has to be inspected and/or large numbers of test plates prepared. In addition a certain level of experience is definitely an asset and probably a requirement.

(4.2) Recommendations for research, development and validation of the system

One important future project will to be find the best treatment schedule and to standardize protocols. This would permit much better comparison of results produced in various laboratories and also lead to development of the most efficient procedures and recording schedules. A larger number of chemicals should be tested using standar-

dized procedures to obtain quantitative data. Such results are sorely needed for development of proper statistical tests. These are essential when the aim is to establish evidence for negative cases, rather than to identify spectacular positive ones.

Addendum: Recent non-reviewed references

The following publications came to our attention after this analysis was completed and have not been considered in the present review: Kappas (1983), Gualandi (1984), Crebelli et al. (1984, 1985), Carere et al. (1985).

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